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DRINKING WATER CRITERIA DOCUMENT

ON

HETEROTROPHIC BACTERIA

DRAFT 5 (May 25, 1984)

HETEROTROPHIC

Prepared by

CRITERIA AND STANDARDS DIVISION
OFFICE OF DRINKING WATER
U.S. ENVIRONMENTAL PROTECTION AGENCY

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16. ABSTRACT

The Office of Drinking Water, U.S. Environmental Protection Agency, has prepared a drinking water criteria document on heterotrophic bacteria. This document includes an extensive review of the following topics: concentration of heterotrophic bacteria in drinking water, health effects, interference with total coliform analysis, utility of heterotrophic bacteria as an indicator of drinking water quality, sampling and analytical procedures.

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I. INTRODUCTION

A. Terminology

Heterotrophic bacteria include all those bacteria which can use organic nutrients for growth. The aquatic environment teems with an extremely diverse flora of these organisms. They include virtually all known primary and secondary bacterial pathogens, whether transient or indigenous, which are spread by the water route. No single analytical tool can satisfactorily detect and enumerate all heterotrophic bacteria or measure their full range of metabolic activities. To study this population, subsets must be created.

One of the most widely used subsets is the "standard plate count." This term is not uniformly used. Other designations include "heterotrophic plate count," "total viable count," "total count," "plate count," "total bacterial count," "bacterial count," "water plate count," "colony count;" also, "aerobic, mesophilic viable bacteria." Standard Methods (APHA, 1980) currently uses the term "Standard Plate Count," but the nomenclature will be changed to "Heterotrophic Plate Count" in the 16th edition of Standard Methods. All of these terms have the same meaning, i.e., the number of bacterial colonies produced on an agar plate under defined medium and incubation conditions. These defined conditions of medium and incubation, as well as other analytical parameters, vary from one country to another, as can be observed in Table I-1 (NATO, in press). The impact that different media, incubation

conditions, and protocol have on the number of bacteria reported will be discussed in Chapter VI. Since procedures vary somewhat, the precise subset of the heterotrophic population measured will also vary somewhat; thus, the heterotrophic count will be defined by the methodology used.

All of the terms just mentioned are misnomers in that they imply an accurate enumeration of all bacterial cells in a water sample. In reality, the plating method permits the growth of only a relatively modest percentage of the organisms present in a sample (Geldreich et al., 1972; NAS, 1977; APHA, 1980; Taylor et al., 1983). This modest percentage only includes aerobic, or facultatively anaerobic, heterotrophic bacteria which find the nutrients in the plating medium to be adequate for growth at the incubation conditions provided. Because the media used are non-selective, some clinically important bacteria cannot be detected by these methods (e.g., Legionella).

The term "Standard Plate Count" has been widely used for decades, and is the term most frequently used in the studies reported in this document. For consistency throughout the rest of this document, this term, abbreviated "SPC," will be used. SPC, when used as an adjective in this document, will refer to the broad class of bacterial types which are detectable by any of the culture procedures commonly used for enumerating heterotrophic bacteria from water.

B. History

Historically, the bacterial plate count occupies a strong position as an analytical tool for determining the microbial quality of a variety of raw and processed food products such as milk, meats, dairy products, and canned foods. It was among the first of the definitive scientific methods employed for quality control in such products, and its use continues today as the major tool for their bacteriological examination. Similarly, the plate count was one of the earliest methods employed for evaluating the bacterial quality of drinking water. In 1891, Miquel recommended the use of the plate count for determination of the sanitary quality of water (Prescott and Winslow, 1904). He provided a table of arbitrary values for grading water on the basis of the number of bacteria per cc, ranging from a count of less than 10 per cc as "excessively pure," to more than 100,000 per cc as very "impure." In 1892, Sternberg (from Geldreich, 1973) proposed that good quality water should contain fewer than 100 bacteria per ml, while waters containing 500 bacteria per ml would be marginal; waters containing over 1000 per ml should be considered as being contaminated with sewage or surface runoff.

The bacterial plate count for analyzing water was used for a number of years, usually in combination with the coliform test, and appeared in 1914 as a U.S drinking water standard (U.S. Treasury Dept., 1914). In this standard, the limit was 100 bacteria per cc.

As experience with the total coliform test and bacterial plate count test accumulated, the fact emerged that the latter provided unreliable information as to the presence of bacterial pathogens in drinking water. For this reason, the test was not included in the succeeding United States Public Health Standards of 1925 and thereafter. While there are currently no Federal requirements for plate counts as a drinking water standard, the USEPA has stated its belief that ". . . the standard plate count is a valid indicator of bacteriological quantity of drinking water, and recommends that it be used in appropriate cases in conjunction with the coliform tests as an operational tool." (40 FR 141, December 24, 1975; p. 59567). The National Academy of Sciences (1977) has stated that "the Standard Plate Count is a valuable procedure for evaluating the bacterial quality of drinking water".

C. Criteria in Other Countries

In Europe, plate counts are widely used for determining water quality (see Table I-1). In South Africa, the recommended limit for total viable organisms is 100 organisms per ml (Grabow, 1977). This is also true in the USSR (Stofen, 1973). The European Communities (1980) has issued the following guide levels for total bacterial counts in drinking water: 10 per ml at 37°C incubation, and 100 per ml at 22°C incubation. Japanese water quality standards include a total colony maximum of 100 organisms per ml (Ministry of Health and Welfare and Japan Water Works Association, 1982).

Table I-1

Total Microbial Count, Drinking Water Analysis[1]

	Canada	FRG	France	Greece	Israel
Total microbial count					
a. Define	Not legally defined, only by the analytical method	Number of colonies/ml developing at fixed temperature and time on defined medium	Number of colonies/ml developing at either 37°C in 24 hours or 20-22°C in 72 hours on defined medium	Number of colonies/ml developing on nutrient agar at 37°C in 24 hrs.	Number of colonies/ml developing on defined medium at 32°C in 72 hours
b. Procedure	Pour plate	Pour plate	Pour plate	Pour plate	Pour Plate
Samples					
a. Volume/plate, ml	As required	1	1	1,0,1 or 1 ml of dilutions	1
b. Number of replicates/ sample	2/dilution	2	2/dilution	2	2
c. Are diluted samples analyzed?	As required	As required	As required	As required	As required
Dilution water if used	Phosphate buffered distilled water	Sterile tap water	Distilled water or Ringer's solution 1/4x	Ringer's solution 1/4x	Phosphate buffered distilled water
Media					
a. Medium	Tryptone glucose extract agar or tryptone glucose yeast agar	Meat extract-peptone agar	yeast extract agar	Nutrient agar	Tryptone glucose yeast agar

-4-a

Total Microbial Count, Drinking Water Analysis - Table I-1 (Continued)

b. Sterilization, time and temperature	15 min.,121°C	20 min.,120°C	20 min.,118°C	15 min.,121°C	15 min.,121°C
Incubation, time and temperature (hours, °C)	48 ± 3, 35 ± 0,5°	44 ± 4, 20 ± 2°	24 ± 1, 37 ± 1° 72 ± 4, 20-22°	24 37°	48 32°
Counting and reporting					
a. Visual aids used	Quebec colony counter	Hand lens (8x)	Hand lens (2-4x)	Hand lens	Quebec colony counter or hand lens (9x)

[1]Source: NATO, 1984.

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Table I-1

Total Microbial (Continued)

	Italy	Netherlands	Norway	Sweden	UK	USA
Total microbial count						
a. Define	Number of colonies/ml developing on tryptic case glucose extract agar	Number of colonies/ml developing on an agar medium under defined conditions	Colony forming units under defined conditions	Number of colonies visible under 1,5x magnification after 48 hours at 22°C on defined medium	Number of organisms growing aerobically and forming colonies under defined conditions	Not required
b. Procedure	Pour plate	Pour plate	Pour plate	Pour plate	Pour plate	
Samples						
a. Volume/plate, ml	1	1	1	1	1	
b. Number of replicates/sample	2	2	2	2	1-2/dilution	
c. Are diluted samples analyzed?	Not required	As required	As required	As required	As required	
Dilution water if used	——	0,1% peptone water	0,9% NaCl	Phosphate buffered distilled water	Ringer's solution 1/4x	
Media						
a. Medium	Tryticase glucose extract agar	Tryptone glucose yeast agar	Tryptone glucose yeast agar	Meat-peptone agar	Yeast extract agar	
b. Sterilization, time and temperature	15 min.,121°C	15 min.,121°C	15 min.,121°C	20 min.,120°C	20 min., 115°C	

Total Microbial - Table I-1 (Continued)

Incubation, time and temperature	24/48 37°C	48,37° 72,22°	72 ± 3, 20 ± 2°	48,22°	24,37° 72, 20-22°
Counting and reporting					
a. Visual aids used	None	Automatic colony counter	Hand lens	Hand lens	Hand lens

D-4-

TABLE I-2

Colony Count; Recommended or Mandatory Values in Europe.[1]

Country	Recommended Value/ml	Mandatory Value/ml	Temperature	Comments
Poland	. . .	25	. . .	
Czechoslovakia	. . .	20	37°C	
	. . .	100	20°C	public supply
	. . .	100	37°C	
	. . .	500	20°C	well water
Yugoslavia	. . .	10	37°C	for treated water
		100	37°C	raw underground water
		300	37°C	raw surface water
Romania	. . .	20	. . .	public water supply for 70,000 consumers
		100 to 300	. . .	other supplies
Switzerland	. . .	100	. . .	raw water entering works
		300	. . .	raw water during distribution
		20	. . .	treated water immediately after treatment
		300	. . .	treated water in distribution system
Spain	. . .	50 to 65	37°C	good quality water
		100	37°C	tolerable quality water
Netherlands, Sweden, GDR	. . .	100	20°C	
Federal Republic of Germany				
Disinfected water	20	. . .	20 ± 2°C	
All other kinds of drinking water	100	. . .	20 ± 2°C	
Water tanks	1000	. . .	20 ± 2°C	
Bottled waters	. . .	1000	20 ± 2°C	
France, Austria, Great Britain	. . .			no guide or imperative values

1 From Muller, 1977

D. Purpose of Document

Currently the drinking water regulations employ total coliforms to indicate water treatment efficiency, contamination in the distribution system, and to signal the presence of fecal contamination in finished water. There is widespread acceptance for using this group as an indicator, but total coliforms are clearly inadequate in some important respects. They are not adequate, for example, for assessing regrowth problems in distribution systems, nor can they adequately signal the presence of some pathogens (e.g., Giardia, viruses, and opportunistic pathogens such as Pseudomonas aeruginosa and Aeromonas hydrophila). Moreover, non-coliforms may interfere with analysis of total coliforms. This document examines the concentration of heterotrophic bacteria in drinking water and the health risk posed by these organisms, evaluates the capacity of heterotrophic bacteria to interfere with total coliform analysis, and discusses the utility of these bacteria as an indicator of drinking water quality.

II. CONCENTRATION OF HETEROTROPHIC BACTERIA IN DRINKING WATER

The number of heterotrophic bacteria in drinking water varies widely, depending on the quality of raw water, efficiency of treatment, condition of the distribution system, and environmental factors. Groundwater sources are usually of a higher sanitary quality than surface sources, especially when undisturbed or protected (Allen and Geldreich, 1978a). A survey of 969 public water supply systems conducted in 1969 by the U.S. Public Health Service found that SPC levels in 76% of raw well water samples were less than 100 organisms/ml (Table II-1) (McCabe et al., 1970). SPC levels in the distribution system, calculated by Geldreich et al. (1972) using the USPHS survey data, were found to be quite low (Table II-2). Forty one percent of the samples were 10/ml or lower and only 11% were over 500/ml. LeChevallier et al. (1980) found the number of SPC bacteria in a distribution system in Oregon ranged from less than 0.02/ml to 10^4/ml.

Numerous environmental factors influence SPC densities. Geldreich et al. (1980) reviewed the types of factors and their effects. Factors include available nutrient type and levels, air and water temperatures, dust, rain, presence of fecal contamination, and depth of reservoir. In the study by LeChevallier et al. (1980), SPC levels were positively correlated with turbidity ($P<0.01$) and temperature ($P<0.05$) and negatively correlated with nitrate ($P<0.05$). In reservoirs, SPC densities vary with season and depth of the water collected. In their study of a raw water reservoir in Kentucky, Geldreich et al. (1980) reported the highest and lowest SPC numbers

occurred during the summer and winter, respectively, at all three water depths examined (Table II-4). Densities were highest at middepth (3-3.6 meters) relative to surface and bottom waters before stratification (July). Once stratification occurred, the highest concentration occurred at the surface. Destratification in October resulted in somewhat higher concentrations in water near the bottom with respect to the other levels. Heterotrophic bacterial density patterns in raw source water impoundments and open finished water reservoirs should be similar.

Chlorination also affects SPC densities. In the study by LeChevallier et al. (1980), dead-end distribution lines in which no free chlorine residual could be detected contained 23 times the number of SPC bacteria found in distribution lines with free residual. Geldreich et al. (1972) examined the effects of chlorination levels on SPC densities and found that a 0.2 mg/l residual generally held SPC concentrations below 500/ml in 98% of distribution system samples (Table II-3). However, some bacterial species tolerate chlorine better than others. Ridgway and Olson (1982) compared the relative chlorine sensitivities of bacteria from a chlorinated and an unchlorinated drinking water distribution system and found that bacteria from the chlorinated system were more resistant to both combined and free forms of chlorine than those from the unchlorinated system. This suggests that chlorinated water may select for chlorine-tolerant organisms.

Table II-1

Bacteriological Quality of Raw Water From Wells

Total Coliform		Fecal Coliform		Plate Count	
Density	Percent Occurrence	Density	Percent Occurrence	Number	Percent Occurrence
0/100 ml	91	0/100 ml	98	0/ml	17
1/100 ml-4/100 ml	6	1/100 ml-4/100 ml	1	1/ml-100/ml	59
5/100 ml-10/100 ml	1	5/100 ml-10/100 ml	<1	101/ml-500/ml	13
11/100 ml-50/100 ml	1	11/100 ml-50/100 ml	<1	501/ml-1,000 ml	4
>50/100 ml	1	>50/100 ml	<1	>1,000/ml	7

McCabe et al. (1970)

Table II-2

Bacterial Plate Count Versus Coliform Detection
in Distribution Water Networks for 969 Community Water Supplies

General Bacterial Population*		Total Coliform		Fecal Coliform	
Density Range per ml	Number of Samples	Occurrences	Percent	Occurrences	Percent
<1-10	1,013	47	4.6	22	2.2
11-30	371	28	7.5	12	3.3
31-100	396	72	18.2	28	7.1
101-300	272	48	17.6	20	7.4
301-500	120	30	25.0	11	9.2
501-1,000	110	21	19.1	9	8.2
>1,000	164	31	18.9	5	3.0
Total	2,446	277		107	

*Standard plate count (48 hr. incubation, 35C).

Geldreich et al. (1972)

Table II-3

Effect of Varying Levels of Residual Chlorine on the Total Plate Count in Potable Water Distribution Systems*

Standard Plate Count†	Residual Chlorine—mg/l							
	0.0	0.01	0.1	0.2	0.3	0.4	0.5	0.6
<1	8.1‡	14.6	19.7	12.8	16.4	17.9	4.5	17.9
1-10	20.4	29.2	38.2	48.9	45.5	51.3	59.1	42.9
11-100	37.3	33.7	28.9	26.6	23.6	23.1	31.8	28.6
101-500	18.6	11.2	7.9	9.6	12.7	5.1	4.5	10.7
501-1,000	5.6	6.7	1.3	2.1	1.8	0	0	0
>1,000	10.0	4.5	3.9	0	0	2.6	0	0
Number of samples	520	89	76	94	55	39	22	28

*Data from a survey of community water supply sytems in nine metropolitan areas.

†Standard plate count (48 hr. incubation, 35C)

‡ All values are percent of samples that had the indicated standard plate count

Geldreich et al. (1972)

Table II-4

Bacterial Characterization of Cruises Creek Reservoir*

1974 Month	Surface			Mid-Depth (3-3.6m)			Bottom (6.1-6.7 m)		
	Fecal Coliforms count/ 100 mL	SPC count/ mL	Temperature °C	Fecal Coliforms count/ 100 mL	SPC count/ mL	Temperature °C	Fecal Coliforms count/ 100 mL	SPC count/ mL	Temperature °C
May	26	390	21.4	55	1000	13.3	80	1200	10.3
June	5.5	1700	26.3	92	2700	16.4	230	2100	11.6
July	3.0	3200	26.6	45	16000	16.7	190	7400	11.1
August	3.4	11000	25.1	58	4900	17.0	190	4000	11.2
September	26	21000	19.2	80	3700	18.2	450	8400	13.8
October	18	530	14.8	21	650	14.8	110	1900	12.7
November	6	180	9.1	4	160	11.8	11	340	11.0

*Reported as geometric mean values
Geldreich et al. (1980)

The SPC population is comprised of a diverse group of heterotrophic microorganisms. Most are gram-negative, nonspore-reforming rods. LeChevallier et al. (1980) isolated about 700 SPC bacteria from drinking water and untreated surface water. These heterotrophic bacteria were completely or partially characterized into 31 different organisms (Table II-5). Over 20% of the organisms recovered from the chlorinated distribution system water from this small Oregon coastal community were Aeromonas and actinomycete species. In this study, 13.5% of the SPC population from the raw water and distribution water during a chlorine failure was gram-positive; 36% of the population in chlorinated drinking water was gram-positive. The SPC profile in Table II-5 is representative of the wide variety of types and varying levels of bacteria during the study period for a particular community.

In contrast to these data, Olson and Hanami (1981) found considerably more gram-positive bacteria from a dead-end of an unchlorinated groundwater system than from a dead-end of a chlorinated surface water system. In this investigation, sampling was accomplished once per quarter for almost 1-1/2 years. Gram-positive bacteria were found in 3 of 7 samples of unchlorinated water and 1 of 7 samples of chlorinated water. Actinomycetes were not found in either system, but were infrequently recovered from some of the wells. Overall, the investigators found that the SPC profile varied

Table II-5

Identification of SPC Bacteria in Distribution Water, Raw Water, and Distribution Water During a Chlorine Failure

Organism	Distribution water		Raw Water		Distribution water during chlorine failure	
	Total*	% of Total	Total	% of Total	Total	% of Total
Actinomycete	37	10.7	0	0	1	1.3
Arthrobacter spp.	8	2.3	2	1.3	7	8.8
Bacillus spp.	17	4.9	1	0.6	0	0
Corynebacterium spp.	31	8.9	3	1.9	0	0
Micrococcus luteus	12	3.5	5	3.2	4	5.0
Staphylococcus aureus	2	0.6	0	0	0	0
S. epidermidis	18	5.2	8	5.1	0	0
Acinetobacter spp.	19	5.5	17	10.8	12	15.0
Alcaligenes spp.	13	3.7	1	0.6	3	3.8
F. meningosepticum	7	2.0	0	0	0	0
Group IVe	4	1.2	0	0	0	0
Group M5	9	2.6	2	1.3	0	0
Group M4	8	2.3	2	1.3	0	0
Moraxella sp.	1	0.3	1	0.6	0	0
Pseudomonas alcaligenes	24	6.9	4	2.5	0	0
P. cepacia	4	1.2	0	0	3	3.8
P. fluorescens	2	0.6	0	0	1	1.3
P. mallei	5	1.4	0	0	0	0
P. maltophilia	4	1.2	9	5.7	4	5.0
Pseudomonas spp.	10	2.9	0	0	0	0
Aeromonas spp.	33	9.5	25	15.9	17	21.3
Citrobacter freundii	6	1.7	8	5.1	1	1.3
Enterobacter agglomerans	4	1.2	18	11.5	7	8.8
Escherichia coli	1	0.3	0	0	0	0
Yersinia enterocolitica	3	0.9	10	6.4	3	3.8
Group IIK biotype I	0	0	1	0.6	8	10.0
Hafnia alvei	0	0	9	5.7	3	3.8
Enterbacter aerogenes	0	0	1	0.6	2	2.5
Enterobacter cloacae	0	0	1	0.6	0	0
Klebsiella pneumoniae	0	0	0	0	2	2.5
Serratia liquefaciens	0	0	1	0.6	0	0
Unidentified	65	18.7	28	17.8	2	2.5
Total	347	100.2	157	99.7	80	100.5

* Number of strains identified for the entire sampling period of one year. LeChavellier, et al. (1980)

with time. By comparing the results of this study and the LeChevallier et al. (1980) study, it is apparent that SPC profiles also may vary as a reflection of source water flora, treatment practices, and distribution characteristics.

In another investigation, Herson and Victoreen (1980) identified bacteria in raw and drinking water in Wilmington, Delaware. SPC bacteria from finished water included species of Enterobacter, Citrobacter, Escherichia, Klebsiella, Pseudomonas, Flavobacterium, Bacillus, Acinetobacter, Arthrobacter, Serratia, Moraxella, Aeromonas, Corynebacterium, and Yersinia. All of these species were also found in the LeChavallier et al. (1980) study.

Armstrong et al. (1982) isolated SPC bacteria from a drinking water facility and from source water and compared the two sets for resistance to six antibiotics. In May and June, 15.8% and 18.2% of the source water bacteria were multiply antibiotic resistant, while 57.1% and 43.5% of bacteria in chlorinated water in the clear well were multiple-antibiotic resistant. Thus, selective pressures (R-factor transfers in treatment basins) can operate at the facility and increase the proportion of multiple-antibiotic resistant bacteria.

Very little is known about the metabolic diversity and activities of most of the organisms in water (Ridgway and Olson,

1982). The National Academy of Sciences (NAS,1982) prepared a list of the effects of selected genera on the quality of drinking water (Table II-6). While some of the microorganisms are not heterotrophic bacteria, the list does convey the wide variety of bacteria which cause degradation activity in drinking water. A few of the bacteria listed are opportunistic pathogens and these are discussed in more detail in Chapter III.

TABLE II-6 Some Genera of Microorganisms Associated with Effects on the Quality of Water in Distribution Systems [1]

Genera	Possible Effect	References [2]
Acinetobacter	Potential indicator antagonist	Geldreich, 1980
Actinomyces	Taste and odors	Geldreich *et al.*, 1972; Larson, 1966
Arthrobacter	Color production (porphyrins); slime formation	Geldreich, 1980; Victoreen, 1969
Bacillus	Nitrate reduction; corrosion potential indicator antagonist	Geldreich *et al.*, 1972
Beggiatoa	"Red" water, sulfur oxidation	Ackerman and Lynde, 1944; Derby, 1947; Larson, 1966; Victoreen, 1974
Crenothrix	"Red" water (iron bacteria)	Ackerman and Lynde, 1944; Arnold, 1936; Larson, 1966
Desulfovibrio	Corrosion; hydrogen sulfide production ("black" water)	Larson, 1966; O'Connor *et al.*, 1975; Russell, 1976
Enterobacter	Regrowth	Geldreich, 1980; Lee and O'Connor, 1975
Escherichia	Fecal indicator	Geldreich *et al.*, 1972
Flavobacterium	Opportunistic pathogen; potential indicator antagonist	Geldreich *et al.*, 1972
Gallionella	"Red" water (iron bacteria)	Larson, 1966; Mackenthun and Keup, 1970; Ridgway and Olson, 1981
Klebsiella	Potential pathogen; regrowth	Geldreich, 1980; Lee and O'Connor, 1975
Leptothrix	"Red" water (iron bacteria)	Geldreich *et al.*, 1972; Larson, 1966; Mackenthun and Keup, 1970
Methylmonas (Methanomonas)	Methane oxidation	Larson, 1966
Micrococcus	Nitrate reduction; corrosion; potential indicator antagonist	Geldreich *et al.*, 1972
Mycobacterium	Potential pathogen	O'Connor *et al.*, 1975
Nitrobacter	Nitrate production; possible corrosion and slime formation	Kooijmans, 1966; Lee and O'Connor, 1975; Russell, 1976
Nitrosomonas	Nitrate production; possible corrosion and slime formation	Kooijmans, 1966; Larson, 1966; Russell, 1976; Victoreen, 1974
Nocardia	Potential pathogen	Water Research Centre, 1977
Proteus	Potential indicator antagonist	Geldreich *et al.*, 1972
Pseudomonas	Opportunistic pathogen; potential indicator antagonist	Geldreich *et al.*, 1972; O'Connor *et al.*, 1975
Serratia	Opportunistic pathogen	Geldreich, 1980
Sphaerotilus	"Red" water	Larson, 1966
Staphylococcus	Potential pathogen	Geldreich, 1980
Streptococcus	Fecal indicator	Ridgway and Olson, 1980
Streptomyces	Taste and odor	American Water Works Association Committee on Tastes and Odors, 1970; Russell, 1976

[1] Source: NAS, 1982

[2] References in NAS, 1982

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III. HEALTH EFFECTS

Between 1946-1982, heterotrophic bacteria implicated in waterborne disease in the U.S. included Salmonella typhi, other Salmonella, Shigella, Campylobacter jejuni, Leptospira, E. coli, Vibrio cholerae, and Yersinia enterocolitica (Lippy and Waltrip, 1984; CDC, 1982; CDC, 1983a). Their primary health effect is gastroenteritis (except for S. typhi). Most of these have a fecal origin and do not proliferate well in water. Other waterborne bacterial primary pathogens include Legionella, Staphylococcus aureus, and Clostridium perfringens. Some of these primary pathogens can grow in media commonly employed for SPC enumeration.

Other heterotrophic bacteria are recognized opportunistic pathogens, i.e., cause disease only in compromised hosts. Many of these organisms can reproduce in water and also grow on conventional SPC media. Opportunistic pathogens commonly present in water are among the major causes of nosocomial infections; the percent of total nosocomial infections they cause increased from 7% during 1961-1965 to 30% during 1976-1979 (Stamm et al., 1981). According to Dixon (1977), at least 5% of those admitted to hospitals get nosocomial infections. About 1% of the affected patients die as a direct result, and for an additional 2.5%, infection is a major factor contributing to death. In a survey of nosocomial infections in 60 hospitals between 1980-1982, the Centers for Disease Control found that of the 103,139 infections reported, 65% were caused by a single pathogen and 20% were caused by multiple pathogens. For

15% of the infections, either cultures were not obtained or laboratory studies did not yield a causative agent. Among the 85% of the infections of known causes, 91% were caused by aerobic bacteria. The 12 most frequently isolated pathogens and the percent they were isolated are found in Table III-1 (CDC, 1983b). Virtually all these organisms can be found in water. Table III-2 is a partial list of opportunistic bacteria which have been implicated in nosocomial infections, and some of the diseases/conditions associated with each. In a sense, this list must be incomplete since any bacteria in the wrong place at the wrong time may cause disease. There is evidence that opportunistic pathogens from the hospital environment can colonize a compromised host and produce an infection when the host's defense further weakens (Schimpff et al., 1972). Contaminated water may serve as a source of these organisms.

Table III-1. The 12 Most Frequently Isolated Pathogens and Their Isolation Rate, 1980-1982

Pathogen	%
E. coli	19.7
S. aureus	10.8
Enterococci	10.1
P. aeruginosa	9.9
Klebsiella spp.	8.0
Proteus spp.	6.1
Enterobacter spp.	5.6
Coagulase-negative staphylococci	4.7
Candida spp.	4.5
Serratia spp.	2.3
Bacteroides spp.	2.1
Group B Streptococcus	1.7
All others	14.7

Source: CDC, 1983b

Table III-2

Opportunistic Pathogens Common in Water

Etiological Agent	Disease	References
Aeromonas hydrophila	septicemia, cellulitis diarrheal disease	Davis et al., 1978; von Graevenitz and Mensch, 1968, Ketover et al., 1973; Rosner, 1964; Hoban and Louie, 1983
Acinetobacter calcoaceticus	meningitis; septicemia; wound, genital, and urinary tract infections	Sonnenwirth, 1973
Alcaligenes faecalis	urinary tract infections, septicemia	Sonnenwirth, 1973; Doxiadis et al., 1960
Chromobacterium violaceum	septicemia; skin ulcers; pneumonia; abscesses in liver, lung, and breast	CDC, 1981; Koburger and May, 1982
Escherichia coli	diarrhea, bacteremia, urinary tract infections	Sonnenwirth, 1973; Greeson, 1981
Flavobacterium	septicemia, pneumonia, meningitis, endocarditis	duMoulin, 1979; von Graevenitz, 1977; Sonnenwirth, 1973
Klebsiella	respiratory and genito-urinary infections, bacteremia, osteomyelitis, meningitis	Brown and Seidler, 1973; Seldon et al., 1971; Vlassoff, 1977
Proteus	urinary tract infections	Sonnenwirth, 1973
Pseudomonas aeruginosa	septicemia, external ear infections, skin rash, diarrhea, wound infections pulmonary infections, gastro-enteritis, burn infections	Hoadley, 1968; Washburn, 1976; Hunter and Ensign, 1947; Wilson et al., 1961; Thomas et al., 1972; Lowbury et al., 1970; Hoadley, 1977 Holder, 1977

Table III-2-- Opportunistic Pathogens (Continued)

Etiological Agent	Disease	References
Serratia marcescens	septicemia, respiratory and urinary tract infections, peritonitis, endocarditis, burn infections	Ringrose et al., 1968; Wilfert et al., 1970 von Graevenitz, 1980
Atypical Mycobacterium	bacteremia, anemia, diarrhea, respiratory tract infections, disseminated infection	Fleischman et al., 1982; Bolan et al., 1983; du Moulin et al., 1983

The portion of opportunistic pathogens among the SPC population may be sizable. LeChevallier et al. (1980) identified the SPC bacteria in source and distribution system water, and found that opportunistic or true pathogens comprised 30% of the total population in chlorinated drinking water samples. These included several Pseudomonas sp., Acinetobacter sp., Klebsiella pneumoniae, Staphylococcus aureus, Moraxella sp., and Serratia liquefaciens. In another study, about half the SPC population in chlorinated drinking water was multiply antibiotic resistant (Armstrong et al., 1982). While there is no published data yet available associating drug resistant organisms in water supplies with a health threat (Sobsey and Olson, 1983), there is evidence that water treatment can select for these organisms (Armstrong et al., 1982).

Some oral infective dose information is available for a few of the primary and opportunistic bacterial pathogens. Much of this data was compiled by the National Academy of Sciences (NAS, 1977) and is reproduced in Table III-3. From this Table, Shigella strains appear to be more pathogenic than the other pathogens listed. For the one opportunistic pathogen listed, enteropathogenic E. coli, an ID_{50} is associated with 10^8 organisms. The oral infective dose for other opportunistic pathogens also appears to be high. Of 12 healthy volunteers fed about 10^7 Klebsiella cells, only one developed serum agglutinins and these were of a low order (Olarte et al., 1961). In another study, three healthy volunteers

Table III-3

Infective Doses For Man Of Bacterial Enteric Pathogens[1]

Enteric Pathogens Dose: Viable Cells	Subjects Infected/Total Tested								
	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	109
Shigella dysenteriae									
Strain M131									
Strain A-1	1/10	2/4	7/10	5/6					
		1/4		2/6					
Shigella flexneri									
Strain 2A#		6/33[a]	33/49[b]	66/87	15/24				
Strain 2A##				1/4	3/4	7/8	13/19	7/8	
Shigella typhi									
Strain Quailes			0/14		32/116		16/32	7/8	
Vibrio cholerae									
Strain Inaba									
With $NaHCO_3$				11/13		45/52		2/2	
No $NaHCO_3$				0/2		0/4	0/4	2/4	1/2
Enteropathogenic *E. coli*									
Strain 4608				0/5		0/5		4/8	

Data from[2]: *Shigella dysenteriae*: Levine, *et al.*, 1973
Shigella flexneri: Dupont, *et al.*, 1972b#
Dupont, *et al.*, 1969##

Salmonella typhi: Hornick, *et al.*, 1970
Vibrio cholerae: Cash, *et al.*, 1974

Enteropathogenic *E. coli*: Dupont, *et al.*, 1971

[a] Dose: 1.8×10^2
[b] Dose: 5×10^3

[1]Source: NAS, 1977
[2]References in NAS, 1977

ingested a suspension of Pseudomonas aeruginosa (up to 10^8 cells). Colonization did not occur in two of the individuals; colonization did occur in the third volunteer ($3x10^6$ cells ingested) after the oral administration of the antibiotic ampicillin (Buck and Cooke, 1969). The results of these two studies support the conclusion of Sears et al. (1950), who found that specific strains of E. coli, when deliberately swallowed in large numbers by healthy individuals, could be recovered in the stool only for limited periods, if at all. This suggests that ingested strains of opportunististic pathogens cannot usually compete successfully with the existing intestinal flora and establish themselves, except when the host is compromised or when drugs (e.g., antibiotics) alter the intestinal flora composition.

Does SPC density correlate with the level of enteric pathogens or incidence of waterborne outbreaks? According to Ptak and Ginsburg (1977), SPC is of limited value for assesing the potential disease-causing ability of drinking water. No relationship between SPC and enteric diseases transmitted by water has been reported (Olivieri, 1983). Geldreich et al. (1972), however, cautions that while no constant relationship appears to exist between SPC levels and the number of pathogens present, it is logical to assume that chance occurrences are proportionally greater as the general bacterial population increases. As an example, Eichhorn (1977) reported that an SPC of over 4000/ml was present in the tap water of a new hospital isolation room,

and species of Pseudomonas (some of whose members are opportunistic pathogens) were the predominant bacteria in this population.

Opportunistic pathogens are commonly isolated from and can grow in drinking water. Hoadley (1977) has reviewed the role of water in the spread of Pseudomonas aeruginosa. According to the review, this organism can be only occasionally isolated from drinking water, especially treated drinking water, unless gross contamination is present. Table III-4 indicates the occurrence of P. aeruginosa in drinking water samples from several studies. Hoadley reports that several investigators have stated that the presence of P. aeruginosa in drinking water is unsatisfactory and must not be ignored.

Pseudomonas species have been implicated in serious nosocomial infections. They can enter hospitals by way of the water supplies and can contaminate faucet and faucet aerators, sinks, baths, washbasin traps, sponges, and humidifier water, from whence they can colonize and infect susceptable hosts (Hoadley, 1977; Cross et al., 1966; Schimpff and Young, 1981). It can grow well in distilled water (Favero et al., 1971). Several studies associating nosocomial infection with Pseudomonas in potable water appear below.

Table III-4

Occurrence of *P. aeruginosa* in Drinking Water Samples

Source of Samples	No. of Samples	No. Positive	Percent Positive
Water supplies in northern Israel	1,000	241	24.1
Water supplies in Germany			
Public water supplies			
ground waters	194	4	2.06
distribution systems	216	16	13.5
Individual water supplies	27	1	3.7
Water supplies in Hungary			
Satisfactory			
well supplies	2,774	31	1.1
spring supplies	123	4	3.3
finished waters	2,785	10	0.4
Unsatisfactory			
well supplies	2,519	54	2.1
spring supplies	47	3	6.4
finished waters	971	14	1.4
Budapest municipal supply			
membrane filter	34,420	88	0.2
most probable number	353	12	3.4
Szeged uncontaminated finished water	374	6	1.6
contaminated finished water	133	46	34.5
Finished waters in southern Ontario	14,486	57	0.4

Hoadley, 1977

In one investigation, Cross et al. (1966) reported that a Pseudomonas species was responsible for four serious postcatheterization wound infections. The source of the organism was traced to a faucet aerator in the cardiac-catheterization laboratory. The authors state that the Pseudomonas species in the aerator was similar in its characteristics to those isolated from the patients, but do not specify the types of tests used for this conclusion.

Wilson et al. (1961), prompted by the death of a premature infant in a hospital nursery by a Pseudomonas aeruginosa infection, found this organism in sinks and faucet aerators. Four of five sinks in the nursery were heavily contaminated with this organism. It was also isolated from four of eight aerators. Other sources were negative, except for the cleaning sponges. The investigators concluded that contaminated tap water was the likely source of the infection.

Du Moulin et al. (1981) reported that a waterborne Pseudomonas sp. was responsible for fouling a closed-circulation hospital air conditioner system. During this period, a Pseudomonas sp. with biochemical characteristics and antibiotic susceptibility patterns identical to the fouling strain became a common isolate in surgical specimens. Seventeen of 371 patients admitted to the respiratory-surgical intensive care unit developed infections due to this Pseudomonas sp. The investigators found that the organism was not entering patient areas via contaminated

water from the air conditioner. Water used in filling the air conditioner was drawn from the domestic water supply of the local community, and water from a hospital water main contained this Pseudomonas sp. at 72 colonies/ml. The investigators inferred that the Pseudomonas sp. in potable water was responsible both for infecting the patients and fouling the air conditioner.

An association between nosocomial infections and the potable water supply has also been observed for nontuberculous mycobacteria. In a study prompted by an outbreak in a hemodialysis center caused by Mycobacterium chelonei (27 of 140 patients), the water treatment system in the dialysis center was found to be heavily contaminated with this organism. This water was used to rinse the dialyzers and also to prepare formaldehyde solution (2%) for disinfecting these devices (Bolan et al., 1983; Carson et al., 1983).

Murphey et al. (1983) isolated Mycobacterium avium-intracellulare (Mai) from 55 patients (2.7% of total), some of whom had respiratory or disseminated infections caused by this organism. While Mai were not recovered from the city water reservoir or treatment plant, they were found in the hospital water entry line and in the 110°F hospital water system. Sputum cultures from volunteers who gargled with hospital water were positive for Mai in 72% of the cases.

Another opportunistic pathogen common in water is Flavobacterium. Herman and Himmelsbach (1965) reported 4 cases of Flavobacterium septicemia following intracardiac operations. An investigation found high densities (2×10^3 to 42×10^6 counts/ml) of this organism in humidifying units (36/55 units positive), drinking fountain bubblers (117/150 positive), distilled water lines (5/10 positive), 37°C water baths (5/7 positive), sink faucets (48/69 positive), and in other areas associated with water. The effect of chlorine residual was examined for 8 Flavobacterium strains during the survey. All were unaffected by 0.8 ppm; 5 of 8 survived 8.0 ppm, while 2 strains remained viable after a 71-day exposure to 100 ppm.

In a survey of 2329 patients in an intensive care unit, duMoulin (1978, 1979) found that the respiratory tracts of 8.4% of the patients were colonized with Flavobacterium species Group IIB during their hospital stay. In 5 cases, the organism was associated with pulmonary infection. In an examination of the hospital environment, this organism was routinely found in tap water, sink drains, ice machines, and in food preparation areas. This strain of Flavobacterium was also found in the reservoir supplying water to the hospital. The results of this study suggested that the ultimate source of the organism was the municipal water supply. duMoulin (1979) reviewed other investigations where Flavobacterium was implicated in human infections, and concluded that in most cases the organism was waterborne.

Nosocomial infections caused by Legionella pneumophila and other Legionella species have also been linked to potable water. There is epidemiologic evidence in a number of reports that showerheads or water taps were associated with these outbreaks (Cordes et al., 1981; Stout et al., 1982; Dufour and Jakobowski, 1982). Legionella is ubiquitous in water (Fliermans et al., 1981) and has been isolated from treated water (Tison and Seidler, 1983). The organism has been found, sometimes at high densities, in hot water tanks (Wadowsky et al., 1982; Plouffe et al., 1983) and this source probably represents the main reservoir for this organism in hospitals and hotels. The aerosal infective dose in guinea pigs is less than 129 organisms (Berendt et al., 1980).

Infections caused by Pseudomonas aeruginosa and Legionella associated with whirlpools, hot tubs, cooling towers and other water receptacles which use water directly from the tap have occasionally occurred (CDC, 1983; Marshall, 1980). Because the link to water-borne agents is tenuous in these cases, this issue will not be pursued in this document.

In recent years, point-of-use devices which claim to remove taste and odor problems and sometimes contaminants from water have been commercially available. Several investigators have demonstrated that with use, the microbial population in devices using activated carbon steadily increases, often resulting in SPC densities in the filter effluent of 10^4-10^5 colonies/ml

(Wallis et al., 1974; Tobin et al., 1981; Fiore and Babineau, 1977). This is particularly true in the first flush after overnight stagnation. Fiore and Babineau (1977) maintained that in their study these high levels were not significantly higher than influent levels after periods of stagnation. In contrast, Tobin et al. (1981) found that SPC densities in effluents after overnight stagnation were as high as 162,000 colonies/ml, with an influent level of 160 colonies/ml. An EPA-supported study compared influent and effluent SPC densities for 25 point-of-use devices, and generally found about a log or more increase in the effluent (U.S. EPA, 1980). Among the opportunistic pathogens which grow on these filters are Pseudomonas aeruginosa and Flavobacterium species (Tobin et al., 1981). The proliferation of these pathogens may pose a health risk to compromised individuals consuming the effluent water.

In summary, opportunistic pathogens may comprise a sizeable portion of the total waterborne SPC population. While this population has not been conclusively implicated in waterborne disease, there is strong evidence that for some nosocomial disease outbreaks this is indeed the case. Oral infective dose data for several waterborne bacterial pathogens are available, but information is virtually nonexistent for the opportunistic pathogens. A positive relationship between SPC densities and waterborne disease outbreaks has been reported in a few cases (Muller, 1977), but published data are sparse.

IV. INTERFERENCE WITH TOTAL COLIFORM ANALYSES

Currently two methods are commonly used for the detection of total coliforms in drinking water: the Multiple Tube Fermentation (MTF) method and the Membrane Filtration (MF) method. Both are subject to interference by high levels of heterotrophic bacteria. In the MTF test, the presence of coliforms is indicated by the production of gas in a lactose-based broth which collects in an inverted tube within a larger test tube (Durham tube). There is evidence that some non-coliforms can produce gas in the Presumptive media either alone or synergistically with other organisms. This causes a false-positive reaction, i.e., the production of gas when no coliforms are present. A more serious type of interference, because standard test results would not indicate the problem exists, occurs when the presence of coliforms is masked by organisms which prevent their growth by the production of toxic substances or by competitive inhibition. This phenomenon is termed a false-negative reaction, and may occur with either the MTF or MF procedures.

Coliform antagonism has been demonstrated by such common aquatic bacteria as Pseudomonas, Micrococcus, Flavobacterium, Bacillus, Actinomyces, Acinetobacter, Arthobacter, Sarcina, and Moraxella (Hutchison et al., 1943; Means and Olson, 1981; Herson and Victoreen, 1980), and these comprised 35% of the SPC bacteria identified by LeChevallier et al. (1980) in chlorinated drinking water. Means and Olson (1981) found that 22.1% of 448 bacterial

isolates from a chlorinated system produced bacteriocin-like substances inhibitory to total coliforms. In their investigation, Flavobacterium/Moraxella represented 57.1% of all bacteria producing these toxic substances. There is also evidence that samples containing high SPC densities reduce coliform numbers during transit to laboratories (McDaniels and Bordner, 1983; Nash and Geldreich, 1981).

The adverse affect of high SPC populations on the MTF test for coliforms was indicated by an investigation in Karachi, Pakistan (Ahmed et al., 1964). In this study, enumeration of Salmonella, Shigella, SPC, and total coliforms in the potable water supply was performed. When the SPC exceeded 500/ml, the Salmonella and Shigella methodology became less sensitive (Table IV-1). These two pathogens were found in 22 of the 44 distribution samples which were negative for coliforms, and in 16 of these 22 (72.7%), SPC levels exceeded 500/ml. It appears that coliforms were suppressed even more than the two pathogens by the high SPC levels.

Seidler et al. (1981) also demonstrated that high SPC levels adversely affect the MFT method. In this study, samples of drinking water were collected from several communities which either disinfected only or practiced no treatment. Total coliform counts were assessed using the MTF technique in Standard Methods (both lactose broth and lauryl tryptose broth) and a more rigorous

Table IV-1

Pathogen Occurrences in 125 Distribution Samples from Karachi Community Supply

Standard Plate Count per ml†	Number of Supplies	Salmonella-Shigella	
		Occurrence	Percent
<1	9	0	00.0
1-500	19	13	68.4
501-1,000	71	33	46.5
>1,000	26	11	42.3

*Selected data from Ahmed et al. (1964) as reported by Geldreich et al. (1972).

†Pour plates incubated at 37C for 48 hours

Table IV-2

Impact of SPC Density in Drinking Water on Coliform Masking in the MPN Technique

SPC Density--colony forming units/mL	Number of Samples	Geometric Mean--Total Coliform Index 100 mL			
		Lauryl Tryptose Broth Presumptive Medium		Lactose Broth Presumptive Medium	
		S-MPN	M-MPN	S-MPN	M-MPN
<10	203	1.2	1.3	1.1	1.5
10 ≤ 49	19	1.4	2.5	1.4	2.9
50 ≤ 99	11	2.1	4.1	1.9	4.0
100 ≤ 249	9	2.7	11.0	2.8	6.5
250 ≤ 500	10	15.1	32.0	6.7	27.0
> 500	10	2.2	14.0	4.2	24.0

Seidler et al., 1981

MFT procedure (M-MPN). Coliform counts from these MFT procedures were compared with the SPC densities in the samples. The data are shown in Table IV-2. As can be noted, coliform counts in all of the MFT techniques used were reduced at SPC densities greater than 500/ml, especially when lauryl tryptose was used.

The same group examined private well and spring water supplies in a rural community of 78 households in Western Oregon (Lamka et al., 1980). Again they found an inverse correlation between SPC densities and coliform incidence, using the MFT technique (Table IV-3).

As mentioned previously, high-background, non-coliform organisms in water samples can also interfere with the MF procedure. SPC organisms can overgrow and obscure or prevent coliform colony formation or reduce sheen formation. To examine this phenomenon, Geldreich et al. (1978) collected 613 samples from flushes of dead-end water mains in Cincinnati. Analyses included MFT, MF, and SPC procedures (Table IV-4). The investigators found that 76 samples contained coliforms by the MFT procedure, but only 19 by the MF procedure. Data analysis demonstrated a correlation between excess SPC densities and desensitization of the MF method. The investigators also surmised that SPC bacteria interfered with the MFT method, since the contents of tubes with heavy growth but no gas did produce gas when inoculated into brilliant green lactose

Table IV-3

Standard Plate Count And The Incidence
Of Total And Fecal Coliform Detection

Standard plate count density range/ 1 ml	No. of samples	Total coliform occur-rences	Total coli-form inci-dence	Fecal coliform occur-rences
<10	61	1	1.6%	1
11-100	177	14	7.9%	3
101-500	52	4	7.7%	0
>500	19	1	5.2%	0

Source: Lamka et al., 1980

Table IV-4

Coliform Occurrences vs Standard Plate Count

	Standard Plate Count Range (per ml)		
	< 500	501-1000	> 1000
All Samples*	502	35	51
Samples with Coliforms** (MPN Method)	50	8	15
Percent of Samples	10%	22.9%	29.4%
MPN Range (per 100 ml)	2-49	5-79	2-920
MPN Mean (per 100 ml)	16	18	110
Samples with Coliforms*** (MF Method)	11	2	5
Percent of Samples	2.2%	5.7%	9.8%
MF Range (per 100 ml)	< 1-18	< 1-50	< 1-90
MF Mean (per 100 ml)	2	7	13
MPN % Occurrence less MF % Occurrence	7.8	17.2	19.6

* 588 of 613 samples had countable SPC
** 3 MPN positive samples did not have countable SPC
*** 1 MF positive samples did not have countable SPC

From Geldreich et al. (1978)

lactose bile broth. They concluded that the MF method, and probably the MFT method, is degraded when SPC densities exceed 500-1000 organisms/ml.

In another study, Geldreich et al. (1972) analyzed bacteria data from distribution water samples from 969 public water supplies in the U.S. They found that the frequency of detecting total and fecal coliforms by the MF procedure increased as the SPC levels increased to 500/ml, but decreased in frequency when SPC levls exceeded 1,000/ml. Their data appear in Table IV-5.

Franzblau et al. (1984) collected 529 water samples from wells and distribution systems, and also found interference with coliform analysis on MF plates at SPC densities exceeding 500 colonies/ ml. At densities above 500/ml, 18 of 37 samples produced overgrowth on the filters and caused false negative results. Anaerobic incubation of MF plates strongly suppressed non-coliform background growth and enhanced coliform recovery by a factor of 2.2.

Clark (1980) examined microbial interference in the Presence-Absence (P-A) test he developed. He enumerated total coliforms in 3254 southern Ontario drinking water samples by both the P-A test and the conventional MF procedure, using m-Endo agar LES. SPC levels were measured after 24 and 48 hours by a modified MF procedure (modified m-PCA) incubated at 35°C. Using SPC results after 48 hours, Clark found no apparent interference with coliform

analysis with either test at SPC densities greater than 1000 colonies/ml (Table IV-6). Interference was demonstrated with the MF procedures, but not the P-A test, when SPC levels were greater than 1000 colonies/ml after 24 hours.

Burlingame et al. (1984), in a laboratory study, examined the effects of pure cultures of four representative SPC bacteria on coliform colony sheen production on membrane filters. All four bacteria were isolated from a municipal water distribution system. A Flavobacterium sp. and a Bacillus sp., which failed to grow on m-Endo coliform medium, did not reduce the number of sheen colonies produced by either E. coli or Enterobacter cloacae, even when added in densities greater than 100,000/filter. Pseudomonas aeruginosa and Aeromonas hydrophila, which are able to grow on m-Endo medium, did interfere with sheen production. Statistical analysis indicated that 3000 P. aeruginosa per filter (30/ml) or 200 A. hydrophila per filter (2/ml) could reduce coliform counts. The investigators concluded this effect was due to overcrowding on the MF plate. Generally, MF plates containing more than 200 colonies are not countable and results are recorded as TNTC (too numerous to count). At the lowest challenge dose found to reduce sheen production, the 200 colony limit was exceeded. The investigators suggest that the coliform interference problem for coliform analyses using the MF procedure may be eliminated by strictly applying this 200 colony limit.

Table IV-5

Bacterial Plate Count vs. Coliform Detection in Distribution Water Networks for 969 Public Water Supplies

General Bacterial Population*		Total Coliform		Fecal Coliform	
Density Range per 1 ml	Number of Samples	Occurrences	Percent	Occurrences	Percent
<1-10	1013	47	4.6	22	2.2
11-30	371	28	7.5	12	3.2
31-100	396	72	18.2	28	7.1
101-300	272	48	17.6	20	7.4
301-500	120	30	25.0	11	9.2
501-1,000	110	21	19.1	9	8.2
>1,000	164	31	18.9	5	3.0
Total	2446	277	—	107	—

*Standard Plate Count (48 hrs. incubation, 35°C)
Geldreich et al., 1972.

The results of this study contrast with the data from other studies mentioned in this section, where interferences were noted on MF plates on which fewer than 200 colonies were observed. This difference may partially be due to coliform injury induced by the SPC population before analysis, resulting in lower coliform counts. Moreover, differences in potency noted between the P. aeruginosa and A. hydrophila in the Burlingame et al., study suggest that other representatives of the SPC population may be sufficiently potent to reduce sheen colonies at densities well below the 200 colony limit.

In summary, these investigations demonstrate that high SPC densities (i.e., over 500/ml) can substantially interfere with both the MFT test and especially the MF test, but that this phenomenon may not occur consistently. One study suggests that interference may not be a significant problem with the Clark P-A test. For the MF procedure, overcrowding on MF plates appears to be a major reason for atypical coliform colonies. Data indicate that this problem may be alleviated (but not eliminated) by anaerobic incubation of plates or a strict application of the 200 colony limit for filters. In addition to interference with coliform analysis methodology, large numbers of SPC bacteria may also reduce coliform levels during sample transit and storage.

Table IV-6

Percentage Frequency of Total Coliforms
Recoveries Based On Two Procedures, and Detection
at Various Density Ranges for Plate Count Occurrences

Count parameter	Counting Range (ml)	No. and percentage of positive samples detected by: Only MF tests[1] No.	%	Only P-A tests[2] No.	%	Combined MF/P-A tests No.	%
24-h, plate count[3]	0	5	0.7	34	4.7	37	5.1
	1-100	118	5.6	223	10.5	251	11.9
	101-1000	52	21.0	68	27.4	80	32.3
	>1000	27	16.9	45	28.1	49	30.6
48-h, plate count[3]	0	—	—	3	1.6	3	1.6
	1-100	72	3.2	178	8.0	200	9.0
	101-1000	79	14.8	116	21.8	129	24.2
	>1000	51	16.6	73	23.8	85	27.2

[1]Membrane filtration procedure using m-Endo LES. Positive sample is 1 coliform/100ml.

[2]Presence-Absence procedure. Positive sample is a presence reaction

[3]m-Standard Plate Count Agar (modified), 35°C incubation

Source: Adopted from Clark (1980).

V. UTILITY OF SPC IN MONITORING DRINKING WATER QUALITY

This chapter examines the use of SPC bacteria as a supplemental surrogate to total coliforms. It will assess the utility of SPC as a general indicator of drinking water quality, and then examine its utility as a surrogate in distribution systems because of deficiencies in the predictive ability of total coliforms.

Large numbers of organisms may pass into the distribution system and proliferate, causing deterioration in water quality. Some of the factors which contribute to this degradation include: 1) inadequate dosage or maintenance of disinfectant, 2) insufficient removal of nutrients and/or turbidity, 3) seasonal temperature fluctuations, 4) extended retention periods in reservoirs, standpipes, and dead-end water mains, and 5) sediment accumulation (Allen et al., 1976). Microbial regrowth can result in slime deposits, development of taste and odor problems, and accelerated pipe deterioration; it may pose a potential health risk and may interfere with total coliform analyses (Allen et al., 1976). More detailed accounts of microbial processes in the distribution system are discussed elsewhere (Ridgway and Olson, 1981; Allen et al., 1980; Geldreich, 1981).

Need for Indicators

The primary reason for evaluating the microbial quality of drinking water is to prevent waterborne outbreaks. Ideally, the detection of specific waterborne pathogens would be the most

direct approach to accomplish this. Unfortunately, in actual practice, this is impossible because of a number of limitations. Pathogens, if present in finished water, are usually present in extremely limited numbers, which would require examination of large-volume samples. Some are so fastidious in their environmental and growth requirements that only highly specialized laboratory techniques may be used to detect them, if they can be detected at all. In addition, extended delays would be involved in carrying out identification procedures, even with the extremely doubtful assumption that every laboratory involved in water testing would have, or could afford, the qualified personnel and other resources to accomplish this.

For these reasons, the direct analysis for pathogen occurrence is rarely employed routinely. Instead, an indirect approach is normally used to signal potential pathogen presence. In this approach, the principal focus for drinking water is on assessing treatment efficiency and on determining whether any significant deterioration of water quality is occurring in the distribution system. An ancillary consideration for drinking water is the detection of fecal contamination. For many decades, tests for total coliform bacteria and turbidity have been used to accomplish these tasks, since these measurements are more reliable and more rapid than detection of specific pathogens.

SPC as an Indicator of Drinking Water Quality

How effective is SPC as a general indicator of drinking water quality? Characteristics of an ideal indicator have been listed by several writers (Bonde, 1963, 1977; National Academy of Sciences, 1977) and they generally include the following:

1. They should be able to monitor treatment efficiency.

2. They should be able to serve as a marker for deterioration of water in the distribution system.

3. They should always be present in sewage and fecally polluted waters when pathogens are present.

4. Their numbers should represent the degree of pollution.

5. They should be present in much greater numbers than the pathogens and therefore still detectable even after great dilution.

6. They should not reproduce in water, or at least, not more than the pathogens.

7. They should have a similar survival time to pathogens in the aquatic environment and in the presence of disinfection and other treatment.

8. They should not be found in unpolluted waters.

9. They should be detectable by simple, inexpensive laboratory tests in a short period of time.

10. They should have stable characteristics.

11. They should be non-pathogenic.

SPC bacteria have many of the above attributes. They are useful in monitoring treatment efficiency and deterioration in the distribution system. They are indigenous to all source waters, are always present in sewage and fecally polluted water, are present in much greater numbers than pathogens and are easily detectable after great dilution. Many members of the SPC population have longer survival times than fecal contaminants in water, and many (e.g., Mycobacterium, Bacillus, and Clostridium) are more resistant to disinfectant than fecal pathogens. SPC bacteria are easily enumerated by inexpensive tests within two days.

Their shortcomings as an indicator are as follows: while SPC populations are generally greater in the presence of pollution,

their numbers do not necessarily represent the degree of pollution. Most species of heterotrophic bacteria, being indigenous to untreated water, reproduce in this habitat and have longer survival times in water than most fecal contaminants. They are found in unpolluted water and tend to have variable characteristics.

The differences between total coliforms and SPC bacteria, with regard to most of the attributes of an ideal indicator, are more a matter of degree. Both total coliforms and SPC bacteria, for example, are present in much greater number than pathogens, but the SPC population is much greater than total coliform levels, providing quantitative information in situations involving greater dilution. Indeed, total coliforms can be considered a subset of the SPC population. In contrast, total coliforms are far more closely associated with fecal pollution and fecal pathogens than SPC bacteria. According to Ptak and Ginsburg (1977), SPC analysis has limited value in assessing the potential disease-causing ability of drinking water, but assumes importance as a harbinger of pollution, as reflected by changes in densities.

There are cases on record where a change in the SPC density has signalled the imminence of a waterborne outbreak. In 1926, for example, Hanover, Germany, experienced flooding of wells by highly contaminated river water. A substantial increase in SPC

numbers was not initially accompanied by positive coliform counts. Hanover experienced 40,000 cases of gastroenteritis followed by an outbreak of typhoid fever (Muller, 1977). Muller (1977) also reported that similar observations occurred at Pforzheim in 1919 and at Gelsenkirchen in 1889.

The most important factor for using SPC bacteria as an indicator is that it can signal deterioration of water quality in the distribution system more reliably than can total coliforms. While some coliform species are capable of proliferation in the distribution system (Ptak et al., 1973; Geldreich, 1983), high densities of SPC bacteria are a more sensitive measure of quality deterioration by virtue of the fact that it can demonstrate higher numbers of total flora.

More specifically, SPC bacteria can be used to monitor deterioration of water quality during and after treatment because it will:

- o provide a method for monitoring the effectiveness of disinfection throughout the distribution system (Geldreich, 1973, 1981).
- o provide warning of a filter bed breakthrough (Ptak and Ginsburg, 1977).

- indicate the existence of sediment accumulation in the distribution system which provides a protective habitat for microorganisms (Geldreich, 1973).

- provide a method for monitoring changes in bacterial quality of finished water storage reservoirs and distribution systems (Geldreich, 1973).

- reflect greater microbial activity leading to consumer complaints, accelerated corrosion, and reduced flow with greater turbulence (Pipes, 1978; Allen and Geldreich, 1978b).

- provide a method for monitoring the condition of activated carbon filters used as end-of-tap treatment devices (Taylor et al., 1979).

- provide information on the likelihood of significant interference with total coliform analysis (Ptak and Ginsburg, 1977) or suppression of coliforms in the distribution system (Means and Olson, 1981).

- signal the likelihood of higher levels of opportunistic pathogens and antibiotic resistant organisms in the water.

A sudden increase in SPC bacteria over baseline values may signal a breakdown in treatment or post-contamination, even in the absence of coliforms, and this increase should be a cause for concern. SPC measurements could also be especially useful in situations where excessive SPC densities might interfere with total coliform analyses or where total coliforms have questionable sanitary significance (Pipes, 1978; Allen and Geldreich, 1975). The latter would include untreated groundwater (Pipes, 1978; Allen and Geldreich, 1975).

At a microbiology workshop sponsored by USEPA's Office of Drinking Water in 1981, a panel of experts concluded that SPC provides an exceptionally sensitive measure of water treatment operation and changes in water quality in the distribution system (Olivieri, 1983). Interestingly, this group ranked SPC and total coliforms equally as surrogates for measuring water deterioration in the distribution system. The panel recommended the use of SPC to monitor the distribution network (along with total coliforms, turbidity, and free chlorine residual), but suggested that a federally-enforceable regulation was not warranted for this group of organisms on the basis of their utility as an indicator. No reason was provided for this latter point of view.

VI. SAMPLING AND ANALYTICAL PROCEDURES

Sampling

Samples should be taken from randomly selected sites of the distribution system. Pipes and Christian (1982) discuss the concept of representative samples and provides a model (Mechanistic Approach) for easy comprehension of this issue. This model demonstrates how to identify sections of a system isolated by the direction of water flow (Figure VI-1). Samples should be collected from each of the isolated segments rather than from different sites from a single isolated section.

Another issue of importance is monitoring frequency. A microbiology panel under the auspices of the NATO Committee on the Challenges of Modern Society (in press) suggested that when SPC is ". . . used routinely to monitor water supplies--at least every three months--it can provide baseline data on the general bacterial population and aid in assessing the degree of bacterial pollution of that supply." An expert panel at a 1978 workshop on indicators of pollution recommended daily monitoring of drinking water (Pipes, 1978). At a microbiology workshop in 1981 sponsored by the USEPA's Office of Drinking Water, four of six panels issued recommendations on monitoring frequency for SPC. Two suggested that SPC bacteria be monitored at a frequency coincident to that of total coliforms (Sobsey and Olson, 1983; Seidler and Evans, 1983). Another panel suggested that SPC be tested "at regular

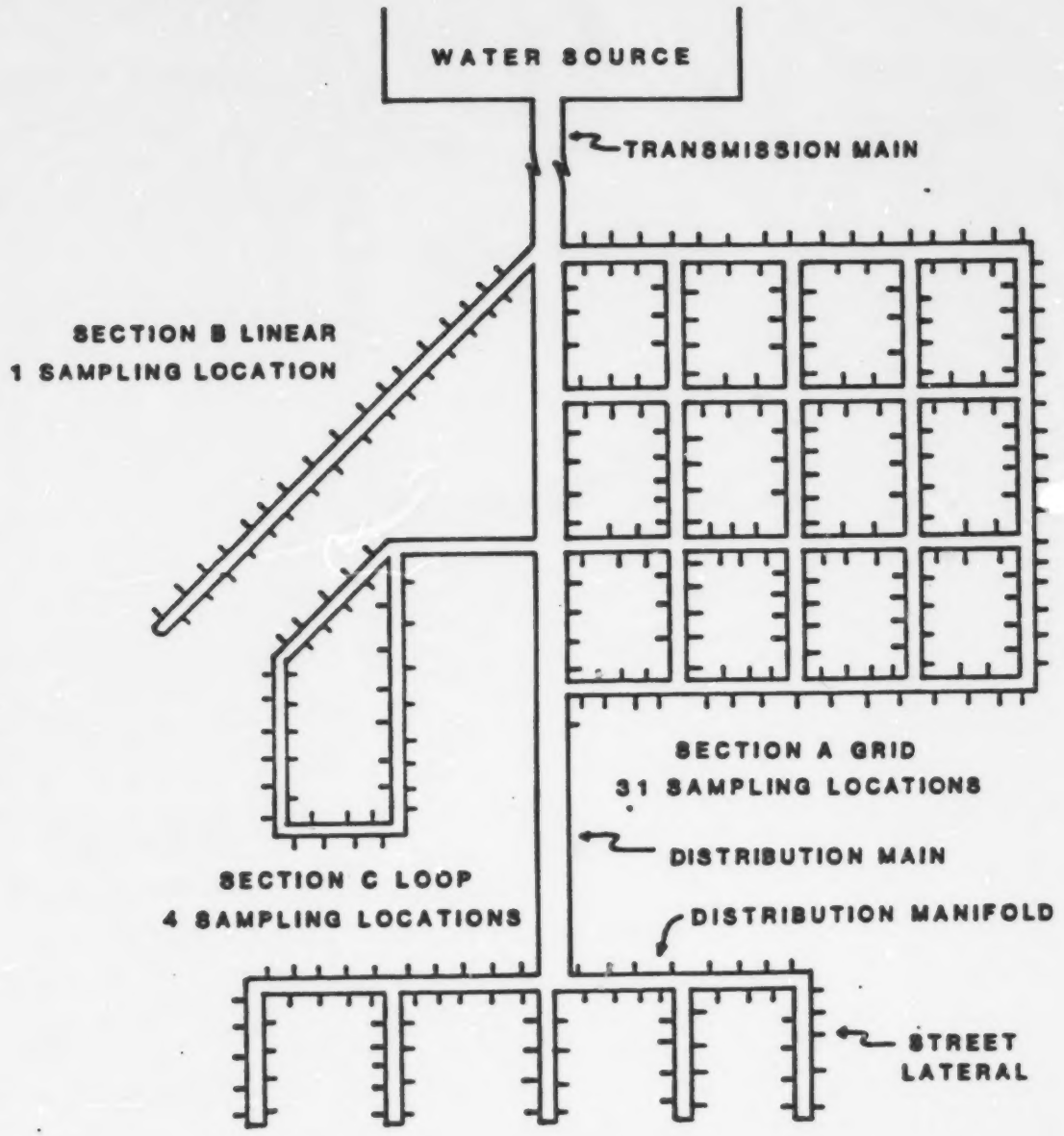

Figure VI-1. Representative Examples of Four Isolated Section Configurations.

Source: Pipes and Christian, 1982.

intervals" (Olivieri, 1983). The fourth panel recommended that SPC be tested for an initial period and on a seasonal basis to establish baseline values, and then tested occasionally thereafter for comparison with the baseline values (Pipes, 1983). The prevailing view, therefore, is that water from the distribution system should be tested for SPC on a frequent basis, at least for an initial time period.

Analytical Procedures

Procedures for measuring heterotrophic bacterial densities in water are empirical because no single growth medium or set of incubation temperatures and times can satisfy the physiological requirements of all heterotrophs in a water sample. Moreover, undercounting will occur when a single colony represents more than one initial bacterium.

Various culture media have been used in Europe and the U.S. for measurement of SPC. Usually they contain peptone, yeast or meat extract, a solidifier (e.g., agar), and sometimes glucose and other nutrients (NATO, Committee on the Challenge of Modern Society, in press). Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1980) has approved two media, Tryptone Glucose Extract Agar and Plate Count Agar. The only difference between them is that one employs beef extract and the other yeast extract.

Bacterial counts also vary markedly based upon medium inoculation procedures. In Standard Methods (APHA, 1980), the only acceptable method for SPC is a pour plate procedure. This has been criticized because the stress of warm agar can cause significant decreases in recovery (Klein and Wu, 1974). The spread plate procedure avoids this problem and provides greater recoveries (van Soestbergen and Lee, 1969; Means et al., 1981; Taylor et al., 1983). A third inoculation procedure, membrane filtration, has recently been developed (Taylor and Geldreich, 1979). These investigators report that the membrane filtration method (m-SPC) provides higher bacterial counts than the conventional pour plate procedure, and also allows much greater volumes of water to be tested than the other methods.

Other media and procedures have been developed recently. Victoreen (1978) reported consistently higher SPC numbers using an eight-fold diluted Standard Methods plate count medium supplemented with iron. Reasoner and Geldreich (1985) also developed a low-nutrient formulation (R-2A) which, in some cases, recovered higher counts than conventional procedures. Reasoner and Geldreich (1981) compared recoveries from a Standard Methods plate count medium, m-SPC medium, and R-2A medium. Inoculation procedures, temperature (20°C, 28°C and 35°C) and time were variables. Optimum conditions for recovery were R-2A spread plates incubated at 28°C for 5-7 days. Means et al. (1981) also compared recoveries on plate count agar

(pour and spread plates), R-2A spread plates, and the m-SPC membrane filtration technique, using water samples from a chlorinated surface water supply and an unchlorinated supply. This group found m-SPC at 35°C yielded higher numbers than R-2A at 20°C after 48 and 96 hours. Both m-SPC and R-2A yielded significantly higher numbers than plate count agar (pour or spread techniques). These studies suggest that the pour plate method in Standard Methods (APHA, 1980) is inferior to alternatives.

In addition to the type of medium used and the medium inoculation procedure employed, incubation time and temperature have a marked effect on SPC recovery. Longer incubation periods at lower temperatures provide higher counts than those currently recommended by Standard Methods (Reasoner and Geldreich, 1981). Table VI-1 shows the incubation conditions used or recommended by selected agencies and laboratories. Incubation temperatures of 20°, 22°, 28°, 35°, and 37°C and incubation times ranging from one to nine days have been employed (Olivieri, 1983). A panel of experts at the microbiology workshop in 1981 has indicated that long incubation times at ambient temperatures are not relevant for the routine monitoring of water, even though higher counts would be anticipated. This is especially true when time is of the essence (Seidler and Evans, 1983).

Other recently developed approaches for measuring SPC include membrane filtration using a special filter apparatus

(Green and Taylor, 1982) and an INT-formazan procedure (Herson and Baker, 1982). The special filter device consists of a filter bonded to an absorbent pad containing dehydrated m-SPC agar. The unit is placed in a chamber containing the water sample for 30 seconds and then removed and incubated (Green and Taylor, 1982). The investigators found that SPC levels were equal to or greater than the conventional membrane procedure using m-SPC agar or the standard pour plate technique. This filter device would be especially useful for large sample analysis. In an investigation of the INT-formazan procedure, Herson and Baker (1982) found that bacterial densities from both distribution water and well water were more than two orders of magnitude greater with the INT-formazan procedure than with the Standard Methods procedure. The INT-formazan procedure can detect contamination of a water supply within 4 hours.

Detailed protocols for conducting conventional SPC analyses can be found elsewhere (e.g., American Public Health Association, 1980; Geldreich, 1975; Bordner and Winter, 1978). For several specifics of detail in the procedure in the U.S. and other countries, see Table VI-1.

Table VI-1

Recommended Or Reported Incubation Temperature And Time For The Bacterial Plate Count For Water Samples

Source	Temperature °C	Time, days
European Economic Community (1975)	37	2
U.S. Environmental Protection Agency (1975)	35	2
German Water Regulations (1975)	20	2
United Kingdom Report # 71	37	1
Water Research Center, United Kingdom (1976)	22 22	3 7
Victoreen (1977)	28	7
Standard Methods (1980)	35	2
Snead, et al. (1980)	20 35	9 4

Source: Olivieri, 1983, amended

VII. SUMMARY AND CONCLUSIONS

There has been much speculation and anecdotal information concerning the direct health risk of consuming high levels of heterotrophic bacteria. There are numerous opportunistic pathogens (and perhaps some frank pathogens) among the SPC population in drinking water, and some of these species have been implicated in disease outbreaks in hospitals, nurseries, nursing homes, and other locations. These opportunistic pathogens include species of Pseudomonas, Flavobacterium, Aeromonas, Klebsiella, Acinetobacter, and a number of other genera. Species of Pseudomonas have been found in hospital sink taps, sink outflows, and faucet aerators in studies prompted by nosocomial infections caused by this genus (Wilson et al., 1961; Cross et al., 1966). Thus, there is evidence to support a direct association between water quality in distribution systems and these outbreaks. The oral infective dose for opportunistic pathogens may be high, perhaps 10^6 - 10^8 organisms or higher, but these data are based on only a few experiments performed with healthy volunteers (Olarte et al., 1961; Buck and Cooke, 1969). Nevertheless, subsets of compromised individuals are undoubtedly susceptible to substantially lower doses of these organisms. In addition, tap water aerosols from showerheads and other plumbing and faucet appurtenances may be a primary route of infection for some infections (Gerba et al., 1975; Dufour and Jakubowski, 1982), and the dose response for inhaled organisms may be lower than an oral dose. While there is no conclusive evidence to date that opportunistic pathogens have

caused disease via the waterborne route, there is strong supportive evidence this is true (see Chapter III). Since virtually everyone in the U.S. is exposed to SPC bacteria whenever they consume or otherwise use potable water, including the compromised population, this is an area of concern.

There are other demonstrable risks, however, regarding high SPC populations in drinking water. Interference with the total coliform analytical procedures--both MTF and MF--was discussed in Chapter IV. A high SPC population can suppress the growth of coliforms, and thereby diminish or destroy their utility in detecting fecal contamination, assessing treatment efficiency, and monitoring water quality deterioration in the distribution system (Seidler et al., 1981; Geldreich et al., 1972). These last two groups of investigators found that total coliform counts were reduced at SPC densities greater than 500 organisms/ml (see data in Chapter IV). There is also strong evidence that drinking water samples containing high SPC levels achieved in regrowth during sample transit reduce the chances of detecting total coliforms upon arrival in the laboratory for processing (McDaniels and Bordner, 1983; Nash and Geldreich, 1981).

In addition to the direct potential health threat of high SPC densities in drinking water, SPC bacteria can be used to reveal water quality deterioration in the treatment plant and in

the distribution system. It can warn when total coliform analysis may be subject to interference. A sudden rise in SPC levels even in the absence of coliforms serves to warn that pathogen breakthrough may be imminent. SPC monitoring can also indicate the level of opportunistic pathogens, as a first approximation. This is one reason SPC levels are more useful than coliform densities in assessing the quality of water from point-of-use devices.

Thus, SPC monitoring may be important for three major reasons: (1) higher densities may reflect higher levels of opportunistic pathogens, (2) higher densities may interfere with total coliform analysis, and (3) they can indicate deterioration of water quality. Total coliform monitoring cannot be used to satisfy point (1) except for opportunistic pathogens which are also coliforms (e.g., Klebsiella pneumoniae). For point (3), SPC monitoring can usefully supplement total coliform monitoring in indicating water deterioration. As indicated previously, Geldreich et al. (1972) and Seidler et al. (1981) suggest that 500 organisms/ml interferes with total coliform analysis, and this value, if used as an upper limit, could control the interference problem and also serve to reduce the possibility of disease via opportunistic pathogens in potable water.

In 1981, the EPA Office of Drinking Water (ODW), in conjunction with the American Society for Microbiology, sponsored a microbiology workshop entitled, "Assessment of Microbiology and Turbidity Standards for Drinking Water". The purpose was to examine options on revising the current drinking water microbiology and turbidity standards, and make recommendations to ODW. Internationally-recognized drinking water experts were assigned to one of six panels, each charged with different topics for discussion. The topics were: (1) Agents of Waterborne Disease, (2) Measurement of Microbial Water Quality, (3) Monitoring, (4) Methods, (5) Source, Treatment and Distribution, and (6) Compliance and Policy Issues. The workshop proceedings were published (Berger and Argaman, 1983). Five panels made recommendations on SPC monitoring and compliance. Two suggested that guidelines be issued, as opposed to regulatory action (Olivieri, 1983; Pipes, 1983). Three others recommended regulations (Sobsey and Olson, 1983; Seidler and Evans, 1983; Greenberg, 1983).

One of the three recommending regulations suggested a monitoring regulation, but felt that a specific density limitation was not feasible, given the variety of circumstances in different systems and seasonal changes in the same system (Seidler and Evans, 1983). This panel offered the following as general guidance: "less than 100/ml, achievable for all systems; 100-500/ml, anticipated during seasonal increases or at certain locations in the system (dead end, low residual) where flushing would be indicated; greater

than 500/ml, poor microbiological quality". The panel also recommended that any sample which showed a 5-10 fold increase over normal levels should prompt an investigation, and that greater than a 10-fold increase should also require reporting.

Another of the three panels which recommended regulations stated that a limit of 500/ml be instituted in locations where complete treatment is not provided for surface water, or disinfection for ground water (Greenberg, 1983). One panel which recommended regulations also suggested as guidance that hospitals, nursing homes, and other institutions with high risk populations be informed of water quality changes in distribution systems that may lead to conditions of increased exposure to opportunistic pathogens within these facilities (Sobsey and Olson, 1983).

Based upon the data presented in this document, a reasonable health goal for SPC densities is 500 colonies/ml. This value is based upon their possible interference with total coliform analysis. Monitoring for this purpose could have the added benefit of providing information about the effectiveness of treatment and about other areas where SPC monitoring has utility. An upper density limit could also reduce the likelihood of hospital-acquired infections, although this premise has not been tested.

The NAS has indicated the importance of SPC monitoring, but has not recommended a specific numerical value. It has, however, stipulated that an SPC density of 500 colonies/ml is attainable by water systems (NAS, 1977). Regulations and guidelines of other countries concerning SPC densities can be found in Chapter I.

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